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Quest for biomarkers of the lean-to-fat ratio by proteomics in beef production

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Producing meat animals with adequate muscular and adipose masses (i.e. lean-to-fat ratio) is an economic challenge for the beef industry. In cattle, conformation (muscle mass) and fatness (adipose tissue mass) are evaluated using the European Union beef carcass classification system (EUROP) scale. The EUROP scale determines the price/kg of carcasses. The lean-to-fat ratio is the result of a dynamic balance between the number and size of muscular and adipose cells, respectively (Bonnet *et al.*, 2010). Identifying proteins that contribute to the increase in the number and volume of adipose and muscular cells has implication for the proposition of biomarkers of growth potential and/or of carcass composition.

The total number of muscle fibres is set by the end of the second trimester of gestation (Picard *et al.*, 2002). Conversely, the number of adipocytes is set by birth or by early adulthood, depending on the anatomical location of the adipose tissue (Vernon, 1986). Thus, we hypothesized that a high throughput molecular characterisation of adipose tissue (AT) and muscle from bovine fetuses differing by the age would be a powerful way to identify proteins associated to the increase in the number and the size of muscular and adipose cells.

We combined measurements of chemical composition, cellularity, histology, enzyme activities, gene expression and proteomics to describe the ontogeny of perirenal AT and *Semitendinosus* muscle in bovine at 60, 110, 180, 210 and 260 days post conception (dpc) in Blond d'Aquitaine (n=3 per age) and Charolais (n=5 per age) breeds. These breeds were chosen for their differences in lean-to-fat ratio in the post-natal life.

Between 110 and 260 dpc (38 and 90% of gestation length, respectively), the increase in the weight of perirenal AT resulted from an increase in the volume and mainly in the number of adipocytes (Taga *et al.*, 2011). The increases in adipocyte volume and number were accompanied by changes in the abundance of 128 proteins among the 143 proteins identified and common to the four last fetal ages studied (Taga *et al.*, 2012). Among the identified proteins, some of them have never been described in the AT and may contribute to hyperplasia of adipose precursors, by controlling cell cycle progression, apoptosis and/or by delaying adipocyte differentiation. The age of 180 dpc seems to be a pivotal age for the transition between proliferation and differentiation of adipocyte progenitors. An increase in the abundance of many proteins involved in differentiation and in the increase in adipocyte volume was observed from 180 dpc.

The fetal growth of semitendinosus muscle was accompanied by changes in the abundance of 245 proteins. We revealed high abundance of proteins involved in apoptosis at 60 and 110 dpc, suggesting that the proliferation – apoptosis balance may play a role in the determination of the total number of fibers (Chaze *et al.*, 2008). The age of 180 dpc corresponds to a reduction in cell proliferation and a transition between the formation of myofibres and their maturation. Indeed, the abundance of stathmin, which has an important role in cell cycle regulation, decreased from 180 dpc onwards. Septin proteins (septin 2 and 11 isoforms) involved in cytoskeletal organisation, scaffolding and cell division plane had a stable expression up to 180 dpc, consistent with intense cell division until this stage, and declined thereafter. Annexin A1, which has an anti proliferative function via the activation of ERK pathways, showed increased abundance from 180 dpc onwards. Increased maturation of fibers from 180 to 260 dpc is reflected by significant changes in the profiles of protein isoforms belonging to metabolic and contractile pathways (Chaze *et al.*, 2009).

The cellular and molecular features of AT and muscle during ontogenesis provide potential hallmarks of adipose and muscular cells hyperplasia or hypertrophy. Among these proteins we hypothesize there are 'master' proteins, thus a current integrative bioinformatic analysis of adipose and muscular data aims to identify them. Then, the relationship between the abundance of these proteins and data from carcass composition will be studied in tissues from meat producing cattle differing by their rearing conditions or by their genotypes.

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